PRODUCT MONOGRAPH

NU-AZATHIOPRINE
Azathioprine Tablets USP
50 mg

IMMUNOSUPPRESSIVE AGENT

Nu-Pharm Inc.
50 Mural Street Units 1 & 2
Richmond Hill, Ontario
L4B 1E4

DATE OF PREPARATION:
January 12, 2004

Control #: 089003

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THERAPEUTIC CLASSIFICATION

Immunosuppressive Agent

ACTIONS AND CLINICAL PHARMACOLOGY

METABOLISM

Azathioprine is well absorbed following oral administration. Maximum serum radioactivity occurs at one to two hours after oral ^{35}S-azathioprine and decays with a half-life of five hours. This is not an estimate of the half-life of azathioprine itself but is the decay rate for all ^{35}S-containing metabolites of the drug. Because of extensive metabolism, only a fraction of the radioactivity is present as azathioprine. Usual doses produce blood levels of azathioprine, and of mercaptopurine derived from it, which are low (<1 µg/mL). Blood levels are of little predictive value for therapy since the magnitude and duration of clinical effects correlate with thiopurine nucleotide levels in tissues rather than with plasma drug levels. Azathioprine and mercaptopurine are moderately bound to serum proteins (30%) and are partially dialyzable.

Azathioprine is cleaved *in vivo* to mercaptopurine. Both compounds are rapidly eliminated from blood and are oxidized or methylated in erythrocytes and liver; no azathioprine or mercaptopurine is detectable in urine after eight hours. Conversion to inactive 6-thiouric acid by xanthine oxidase is an important degradative pathway, and the inhibition of this pathway in patients receiving Zyloprim® (allopurinol) is the basis for the azathioprine dosage reduction required in these patients (see Drug Interactions under PRECAUTIONS). Proportions of metabolites are different in individual patients, and this presumably accounts for variable magnitude and duration of drug effects. Renal clearance is probably not important in predicting biological effectiveness or toxicities, although dose reduction is practised in patients with poor renal function.

In view of the observations by Schwartz *et al* that mercaptopurine suppressed the antibody response in rabbits injected with bovine serum albumin, the effects of azathioprine on the formation of antibodies were investigated. In the suppression of the formation of antibodies in mice to sheep red cells, as determined by hemagglutinin titres, azathioprine was found to be

superior to mercaptopurine. Whereas mercaptopurine was active only at its maximum tolerated dose of 75 mg/kg, azathioprine was active at 25 mg/kg and was tolerated in doses up to 60 mg/kg for the dosage schedule employed (intraperitoneal injection for 4 successive days beginning at the time of the antigenic stimulus). The anti-immune effects of azathioprine are not due entirely to the mercaptopurine derived therefrom by splitting *in vivo*.

Another line of evidence which suggests that part of the activity of azathioprine may be due to its reaction with sulfhydryl compounds is the potentiation of its anti-immune effect by the simultaneous administration of Myleran® (busulfan). (Busulfan is also known to react with sulfhydryl groups in tissues.) Thus the combination of azathioprine (10 mg/kg) and busulfan (30 mg/kg) produced a marked suppression of the antibody response, whereas the minimum effective dose of azathioprine alone is 25 mg/kg; busulfan alone is inactive at its maximum tolerated dose of 40 mg/kg. The combination of mercaptopurine (25 mg/kg) and busulfan (25 mg/kg) is inactive.

HOMOGRAFT SURVIVAL

Although the use of azathioprine for inhibition of renal homograft rejection is well established, the mechanism(s) for this action are somewhat obscure. The drug suppresses hypersensitivities of the cell-mediated type and causes variable alterations in antibody production. Suppression of T-cell effects, including ablation of T-cell suppression, is dependent on the temporal relationship to antigenic stimulus or engraftment. This agent has little effect on established graft rejections or secondary responses.

Alterations in specific immune responses or immunologic functions in transplant recipients are difficult to relate specifically to immunosuppression by azathioprine. These patients have subnormal responses to vaccines, low numbers of T-cells, and abnormal phagocytosis by peripheral blood cells, but their mitogenic responses, serum immunoglobulins and secondary antibody responses are usually normal.

IMMUNOINFLAMMATORY RESPONSE

Azathioprine suppresses disease manifestations as well as underlying pathology in animal models of autoimmune disease. For example, the severity of adjuvant arthritis is reduced by azathioprine.

The mechanisms whereby azathioprine affects autoimmune diseases are not known. Azathioprine is immunosuppressive, delayed hypersensitivity and cellular cytotoxicity tests being suppressed to a greater degree than are antibody responses. In the rat model of adjuvant arthritis, azathioprine has been shown to inhibit the lymph node hyperplasia which precedes the onset of the signs of the disease. Both the immunosuppressive and therapeutic effects in animal models are dose-related. Azathioprine is considered a slow-acting drug and effects may persist after the drug has been discontinued.

COMPARATIVE BIOAVAILABILITY

Two comparative bioavailability studies were performed on healthy human volunteers - one fasted and one fed study. The rate and extent of absorption of 6-mercaptopurine were measured and compared following oral administration of 50 mg of either NU-AZATHIOPRINE 50 mg tablets or Imuran 50 mg tablets. The results from measured data are summarized as follows.

FASTED STUDY:

Summary Table of the 6-Mercaptopurine Comparative Bioavailability Data (1 × 50 mg) From Measured Data

Parameter	Geometric Mean Arithmetic Mean (C.V.)		Ratio of Geometric Means (%)
	NU-AZATHIOPRINE	**Imuran®†**	
AUC_{0-T} (ng·hr/mL)	27.88 31.59 (52.42)	28.96 30.86(34.64)	96.3
$AUC_{0-\infty}$ (ng·hr/mL)	33.95 37.31 (45.10)	34.61 36.45(31.33)	98.1
C_{max} (ng/mL)	15.3 18.10 (63.20)	16.9 18.75(42.09)	90.5
T_{max} (hours)*	1.33 (71.41)	1.25 (66.65)	-
$T_{1/2}$ (hours)*	1.51 (35.22)	1.52 (31.50)	-

* Arithmetic means only (CV%).
† Imuran® is manufactured by Glaxo Wellcome Inc., and was purchased in Canada.

FED STUDY:

Summary Table of the 6-Mercaptopurine Comparative Bioavailability Data (1 × 50 mg) From Measured Data

Parameter	Geometric Mean Arithmetic Mean (C.V.)		Ratio of Geometric Means (%)
	NU-AZATHIOPRINE	Imuran®†	
AUC_{0-T} (ng·hr/mL)	16.1078 17.737 (44.9)	17.6405 18.998(39.0)	91.31
$AUC_{0-\infty}$ (ng·hr/mL)	23.6023 24.763 (31.8)	22.7782 24.102(34.5)	103.62
C_{max} (ng/mL)	6.9298 8.299 (69.8)	7.8071 8.868 (54.4)	88.76
T_{max} (hours)*	1.847 (59.6)	1.791 (54.0)	-
$T_{1/2}$ (hours)*	2.216 (31.2)	2.075 (43.2)	-

* Arithmetic means only (CV%).
† Imuran® is manufactured by Glaxo Wellcome Inc., and was purchased in Canada.

INDICATIONS AND CLINICAL USE

CHRONIC IMMUNOSUPPRESSION WITH THIS PURINE ANTIMETABOLITE MAY INCREASE RISK OF NEOPLASIA. PHYSICIANS USING THIS DRUG SHOULD BE VERY FAMILIAR WITH THIS RISK AS WELL AS WITH POSSIBLE HEMATOLOGIC TOXICITIES. SEE BELOW UNDER WARNINGS.

RENAL HOMOTRANSPLANTATION

Azathioprine is indicated as an adjunct for the prevention of rejection in renal homotransplantation. Experience with over 16,000 transplants shows a five-year patient survival of 35% to 55%, but this is dependent on donor, match for HLA antigens, antidonor or anti B-cell alloantigen antibody and other variables. The effect of azathioprine on these variables has not been tested in controlled trials.

RHEUMATOID ARTHRITIS

Azathioprine is indicated only in adult patients meeting criteria for classic or definite rheumatoid arthritis as specified by the American Rheumatism Association. Azathioprine should be restricted to patients with severe, active and erosive disease not responsive to conventional management including rest, acetylsalicylic acid or other nonsteroidal drugs, or to agents in the class of which gold is an example. Rest, physiotherapy and salicylates should be continued while azathioprine is given, but it may be possible to reduce the dose of corticosteroids in

patients on azathioprine. The combined use of azathioprine with gold, antimalarials or penicillamine has not been studied for either added benefit or unexpected adverse effects. The use of azathioprine with these agents cannot be recommended.

CONTRAINDICATIONS

Azathioprine should not be given to patients who have shown hypersensitivity to the drug.

Patients with rheumatoid arthritis previously treated with alkylating agents (cyclophosphamide, chlorambucil, melphalan or others) may have a prohibitive risk of neoplasia if treated with azathioprine.

Azathioprine should not be used in treating rheumatoid arthritis in pregnant women. Azathioprine should not be used to treat children with rheumatoid arthritis.

WARNINGS

Severe leukopenia and/or thrombocytopenia may occur in patients on azathioprine. Macrocytic anemia and severe bone marrow depression may also occur. Hematologic toxicities are dose related and may be more severe in renal transplant patients whose homograft is undergoing rejection. It is suggested that patients on azathioprine have complete blood counts, including platelet counts, weekly during the first month, twice monthly for the second and third months of treatment, then monthly or more frequently if dosage alterations or other therapy changes are necessary. Delayed hematologic suppression may occur. Prompt reduction in dosage or temporary withdrawal of the drug may be necessary if there is a rapid fall in, or persistently low leukocyte count or other evidence of bone marrow depression. Leukopenia does not correlate with therapeutic effect; therefore, the dose should not be increased intentionally to lower the white blood cell count.

Serious infections are a constant hazard for patients receiving chronic immunosuppression, especially for homograft recipients. Fungal, viral, bacterial and protozoal infections may be fatal and should be treated vigorously. Reduction of azathioprine dosage and/or use of other drugs should be considered.

Azathioprine is mutagenic in animals and humans, carcinogenic in animals, and may increase the patient's risk of neoplasia. Renal transplant patients are known to have an increased risk of malignancy, predominantly skin cancer and reticulum cell or lymphomatous tumours. The risk of post-transplant lymphomas may be increased in patients who receive aggressive treatment with immunosuppressive drugs. The degree of immunosuppression is determined not only by the immunosuppressive regimen, but also by a number of other patient factors. The number of immunosuppressive agents may not necessarily increase the risk of post-transplant lymphomas. However, transplant patients who receive multiple immunosuppressive agents may be at risk for over-immunosuppression; therefore, immunosuppressive drug therapy should be maintained at the lowest effective levels. Information is available on the spontaneous neoplasia risk in rheumatoid arthritis, and on neoplasia following immunosuppressive therapy of other autoimmune diseases. It has not been possible to define the precise risk of neoplasia due to azathioprine. The data suggest the risk may be elevated in patients with rheumatoid arthritis, though lower than for renal transplant patients. However, acute myelogenous leukemia as well as solid tumours have been reported in patients with rheumatoid arthritis who have received azathioprine. Data on neoplasia in patients receiving azathioprine can be found under ADVERSE REACTIONS.

Azathioprine has been reported to cause temporary depression in spermatogenesis and reduction in sperm viability and sperm count in mice at doses 10 times the human therapeutic dose; a reduced percentage of fertile matings occurred when animals received 5 mg/kg.

A persistent negative nitrogen balance has been observed in some patients on continuous azathioprine dosage; if this should occur, the dose should be reduced as this has been found to correct the situation.

USE IN PREGNANCY

Azathioprine can cause fetal harm when administered to a pregnant woman.

Azathioprine should not be given during pregnancy or in patients of reproductive potential without careful weighing of risk versus benefit. Use of azathioprine in pregnant patients should be avoided whenever possible. If this drug is used during pregnancy or if the patient becomes pregnant while taking this drug, the patient should be apprised of the potential harm to the fetus. Women of child-bearing age should be advised to avoid becoming pregnant.

Azathioprine is teratogenic in rabbits and mice when given in doses equivalent to the human dose (5 mg/ kg/daily). Abnormalities included skeletal malformations and visceral anomalies.

There are no adequate and well-controlled studies in pregnant women.

Limited immunologic and other abnormalities have occurred in a few infants born of renal allograft recipients on azathioprine. In a detailed case report, documented lymphopenia, diminished IgG and IgM levels, CMV infection, and a decreased thymic shadow were noted in an infant born to a mother receiving 150 mg azathioprine and 30 mg prednisone daily throughout pregnancy. At 10 weeks most features were normalized. DeWitte *et al* reported pancytopenia and severe immune deficiency in a preterm infant whose mother received 125 mg azathioprine and 12.5 mg prednisone daily. There have been two published reports of abnormal physical findings. Williamson and Karp described an infant born with preaxial polydactyly whose mother received azathioprine 200 mg daily and prednisone 20 mg every other day during pregnancy. Tallent *et al* described an infant with a large myelomeningocele in the upper lumbar region, bilateral dislocated hips, and bilateral talipes equinovarus. The father was on long-term azathioprine therapy.

PRECAUTIONS

GENERAL

The dosage that will be tolerated or effective will vary from patient to patient. Therefore, careful management is necessary to obtain the optimum therapeutic effect and to reduce toxicity. Caution must be exercised to observe early signs of depression of the bone marrow which may result in leukopenia and eventually thrombocytopenia and bleeding. Since this drug may have a delayed action, it is important to withdraw the medication temporarily at the first sign of an abnormally large fall in the white cell count or of abnormal depression of the bone marrow. It must be kept in mind that patients with impaired renal function may have slower elimination of the drug and a greater cumulative effect. Lower dose if there is impaired renal function. It is recommended that the drug be withheld if there is evidence of toxic hepatitis or biliary stasis.

HEMATOLOGIC

There are rare individuals with an inherited deficiency of the enzyme thiopurine methyltransferase (TPMT) who may be unusually sensitive to the myelosuppresive effect of azathioprine and prone to developing rapid bone marrow suppression following the initiation of treatment with azathioprine.

GASTROINTESTINAL

A gastrointestinal hypersensitivity reaction characterized by severe nausea and vomiting has been reported. These symptoms may also be accompanied by diarrhea, rash, fever, malaise, myalgias, elevations in liver enzymes, and occasionally, hypotension. Symptoms of gastrointestinal toxicity may often develop within the first several weeks of azathioprine therapy and are reversible upon discontinuation of the drug. The reaction can recur within hours after rechallenge with a single dose of azathioprine.

DRUG INTERACTIONS

Allopurinol

The principal pathway for detoxicification of azathioprine is inhibited by allopurinol. In patients receiving azathioprine, the concomitant administration of Zyloprim® (allopurinol) will require a reduction in dose to approximately 1/3 to 1/4 of the usual dose of azathioprine. Subsequent adjustment of doses of azathioprine should be made on the basis of therapeutic response and any toxic effects.

Other Agents Affecting Myelopoesis

Drugs which may affect leukocyte production, including cotrimoxazole, may lead to exaggerated leukopenia, especially in renal transplant recipients.

Angiotensin Converting Enzyme Inhibitors

The use of angiotension converting enzyme inhibitors to control hypertension in patients on azathioprine has been reported to induce anemia and severe leukopenia.

Warfarin

Azathioprine may inhibit the anticoagulant effect of warfarin.

Non-Depolarizing Muscle Relaxants

There is clinical evidence that azathioprine antagonizes the effect of non-depolarizing muscle relaxants such as curare, d-tubocurarine and pancuronium. Experimental data confirm that azathioprine reverses the neuromuscular blockade caused by d-tubocurarine, and show that azathioprine potentiates the neuromuscular blockade caused by succinylcholine.

USE IN PREGNANCY

See WARNINGS.

USE IN NURSING MOTHERS

The use of azathioprine in nursing mothers is not recommended. Azathioprine or its metabolites are transferred at low levels, both transplacentally and in breast milk. Because of the potential for tumorigenicity shown for azathioprine, a decision should be made on whether to discontinue nursing or discontinue the drug, taking into account the importance of the drug to the mother.

USE IN CHILDREN

Safety and efficacy of azathioprine in children have not been established.

INFORMATION FOR PATIENTS

Patients being started on azathioprine should be informed of the necessity of periodic blood counts while they are receiving the drug and should be encouraged to report any unusual bleeding or bruising to their physician. They should be informed of the danger of infection while receiving azathioprine and asked to report signs and symptoms of infection to their physician. Careful dosage instructions should be given to the patient, especially when azathioprine is being administered in the presence of impaired renal function or concomitantly with allopurinol (see Drug Interactions and DOSAGE AND ADMINISTRATION). Patients should be advised of the potential risks of the use of azathioprine during pregnancy and during the nursing period. The increased risk of neoplasia following azathioprine therapy should be explained to the patient.

ADVERSE REACTIONS

The principal and potentially serious toxic effects of azathioprine are hematologic and gastrointestinal. The risks of secondary infection and neoplasia are also significant (see WARNINGS). The frequency and severity of adverse reactions depend on the dose and duration of azathioprine as well as on the patient's underlying disease or concomitant therapies. The incidence of hematologic toxicities and neoplasia encountered in groups of renal homograft recipients is significantly higher than that in studies employing azathioprine for rheumatoid arthritis. The relative incidences in clinical studies are summarized below:

Toxicity	Renal Homograft	Rheumatoid Arthritis
Leukopenia		
Any Degree	>50%	28%
<2500/mm^3	16%	5.3%
Infections	20%	<1%
Neoplasia		*
Lymphoma	0.5%	
Others	2.8%	

*Data on the rate and risk of neoplasia among persons with rheumatoid arthritis treated with azathioprine are limited. The incidence of lymphoproliferative disease in patients with RA appears to be significantly higher than that in the general population. In one completed study, the rate of lymphoproliferative disease in RA patients receiving higher than recommended doses of azathioprine (5 mg/kg/day) was 1.8 cases per 1000 patient years of follow-up, compared with 0.8 cases per 1000 patient years of follow-up in those not receiving azathioprine. However, the proportion of the increased risk attributable to the azathioprine dosage or to other therapies (i.e., alkylating agents) received by patients treated with azathioprine cannot be determined.

HEMATOLOGIC

Leukopenia and/or thrombocytopenia are dose dependent and may occur late in the course of azathioprine therapy. Dose reduction or temporary withdrawal allows reversal of these toxicities. Infection may occur as a secondary manifestation of bone marrow suppression or leukopenia, but the incidence of infection is 30 to 60 times greater in renal homotransplantation than in rheumatoid arthritis. Macrocytic anemia and/or bleeding have been reported in patients on azathioprine.

GASTROINTESTINAL

Nausea and vomiting may occur within the first few months of azathioprine therapy, and occurred in approximately 12% of 676 rheumatoid arthritis patients. The frequency of gastric disturbance can often be reduced by administration of the drug in divided doses and/or after meals. However, in some patients, nausea and vomiting may be severe and may be accompanied by symptoms such as diarrhea, fever, malaise, and myalgias (see PRECAUTIONS). Vomiting with abdominal pain may occur rarely with a hypersensitivity pancreatitis.

HEPATIC

Hepatotoxicity manifest by elevation of serum alkaline phosphatase, bilirubin and/or serum transaminases is known to occur with thiopurines including azathioprine and Purinethol® (6-mercaptopurine). This toxic hepatitis with biliary stasis is known to occur in homograft

recipients. Hepatotoxicity has been uncommon in rheumatoid arthritis patients on azathioprine (less than 1%). Hepatotoxicity following transplantation most often occurs within 6 months of transplantation and is generally reversible after interruption of azathioprine. A rare, but life-threatening hepatic venoocclusive disease associated with chronic administration of azathioprine has been described in transplant patients and in one patient receiving azathioprine for panuveitis. Periodic measurement of serum transaminases, alkaline phosphatase, and bilirubin is indicated for early detection of hepatotoxicity. If hepatic venoocclusive disease is clinically suspected, azathioprine should be permanently withdrawn.

OTHERS

Additional side effects of low frequency have been reported. These include skin rashes, alopecia, fever, arthralgias, diarrhea, steatorrhea and negative nitrogen balance and reversible interstitial pneumonitis.

SYMPTOMS AND TREATMENT OF OVERDOSAGE

Initial symptoms are nausea and vomiting; and symptoms appearing later are leukopenia, thrombocytopenia, hepatic necrosis and anorexia.

For the treatment of overdosage, administer gastric lavage and fluids; blood transfusions may be needed due to suppression of the proliferation of granulocytes.

About 30% of azathioprine is bound to serum proteins, but approximately 45% is removed during an 8-hour hemodialysis. A single case has been reported of a renal transplant patient who ingested a single dose of 7500 mg azathioprine. The immediate toxic reactions were nausea, vomiting and diarrhea, followed by mild leukopenia and mild abnormalities in liver function. The white blood cell count, SGOT and bilirubin returned to normal 6 days after the overdose.

DOSAGE AND ADMINISTRATION

RENAL HOMOTRANSPLANTATION

The dose of azathioprine required to prevent rejection and minimize toxicity will vary with individual patients; this necessitates careful management. The initial dose is usually 3-5 mg/kg daily, beginning at the time of transplant. Azathioprine is usually given as a single daily dose on

the day of, and in a minority of cases one to three days before, transplantation. Azathioprine is often initiated with the intravenous administration of the sodium salt, with subsequent use of tablets (at the same dose level) after the post-operative period. Intravenous administration of the sodium salt is indicated only in patients unable to tolerate oral medications. Dose reduction to maintenance levels of 1-3 mg/kg daily is usually possible. The dose of azathioprine should not be increased to toxic levels because of threatened rejection. Discontinuation may be necessary for severe hematologic or other toxicity, even if rejection of the homograft may be a consequence of drug withdrawal.

RHEUMATOID ARTHRITIS

Azathioprine is usually given on a daily basis. The initial dose should be approximately 1.0 mg/kg (50-100 mg) given as a single dose or on a twice daily schedule. The dose may be increased, beginning at six to eight weeks and thereafter by steps at four-week intervals, if there are no serious toxicities and if initial response is unsatisfactory. Dose increments should be 0.5 mg/kg daily, up to a maximum dose of 2.5 mg/kg/day. Therapeutic response occurs after several weeks of treatment, usually six to eight; an adequate trial should be a minimum of 12 weeks. Patients not improved after twelve weeks can be considered refractory. Azathioprine may be continued long-term in patients with clinical response, but patients should be monitored carefully, and gradual dosage reduction should be attempted to reduce risk of toxicities. Maintenance therapy should be at the lowest effective dose, and the dose given can be lowered, with decremental changes of 0.5 mg/kg or approximately 25 mg daily every four weeks while other therapy is kept constant. The optimum duration of maintenance azathioprine has not been determined. Azathioprine can be discontinued abruptly, but delayed effects are possible.

USE IN RENAL DYSFUNCTION

Relatively oliguric patients, especially those with tubular necrosis in the immediate post-cadaveric transplant period, may have delayed clearance of azathioprine or its metabolites, or be particularly sensitive to this drug and are usually given lower doses.

PHARMACEUTICAL INFORMATION

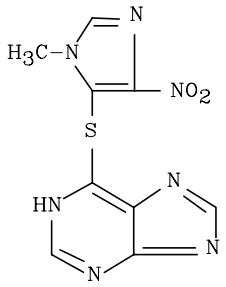
DRUG SUBSTANCE

Common Name: Azathioprine

Chemical Names: 1) 1*H*-Purine,6-[(1-methyl-4-nitro-1*H*-imidazol-5-yl)-thio]-;

2) 6-[(1-Methyl-4-nitro-1*H*-imidazol-5-yl)thio]-1*H*-purine.

Structural Formula:



Molecular Formula: $C_9H_7N_7O_2S$

Molecular Weight: 277.27

pKa: 8.2 (25°C)

Melting Point: Approximately 238°C with decomposition.

Description:

Azathioprine is a pale yellow, odourless powder. It is insoluble in water, soluble in dilute solutions of alkali hydroxides, sparingly soluble in dilute mineral acids and very slightly soluble in alcohol and in chloroform.

COMPOSITION

In addition to azathioprine, each NU-AZATHIOPRINE Tablet contains the non-medicinal ingredients lactose, magnesium stearate, microcrystalline cellulose and starch.

STABILITY AND STORAGE RECOMMENDATIONS

Store at room temperature (15-30°C), protected from light.

SPECIAL INSTRUCTIONS

Tablets should be returned to the manufacturer for destruction. Proper precautions should be taken in packaging these materials for transport.

All materials which have come in contact with cytotoxic drugs should be segregated and incinerated at 1000°C or more. Sealed containers may explode.

Personnel regularly involved in the preparation and handling of cytotoxic agents should have biannual blood examinations.

AVAILABILITY OF DOSAGE FORMS

NU-AZATHIOPRINE (azathioprine) 50 mg Tablets are pale yellow, peanut-shaped tablets, scored and engraved "AZ 50" on one side and “APO” on the other. NU-AZATHIOPRINE Tablets are available in bottles of 100 tablets.

TOXICOLOGY

Acute toxicity studies in mice and rats showed a species variation and a somewhat lower toxicity when azathioprine was administered orally than when it was given intraperitoneally. The single LD_{50} dose in mice is 650 mg/kg, intraperitoneally, and about 2500 mg/kg, orally. In rats, the single LD_{50} is 310 mg/kg, intraperitoneally, and 400 mg/kg, orally.

Death after an LD_{50} dose, and even after an LD_{100} dose, was delayed two to seven days. Subacute toxicity studies also demonstrated the cumulative toxicity.

When the drug was given to mice for five successive days, the maximum tolerated daily dose was 100 mg/kg intraperitoneally and 200 mg/kg orally. In rats given five consecutive daily doses, the LD_{50} was 100 mg/kg whether the drug was given intraperitoneally or orally, and in these animals death occurred within a day or two of the last dose.

Chronic toxicity studies in rats revealed that all the animals that died of drug toxicity at the two highest dosage levels (60 mg/kg body weight/day and 180 mg/kg body weight/day incorporated in the diet) showed agranulocytic spleens and bone marrows and hemorrhagic lungs.

There was also some colloid depletion of the thyroid and failure of spermatogenesis. None of the animals that survived the six-month period showed blood dyscrasias or histological abnormalities.

Dogs receiving 1 or 2 mg/kg body weight/day orally for 18 weeks showed a normal weight gain and no hematologic changes. Of four dogs receiving 4 mg/ kg/day orally for 18 weeks, two had episodes of fever during the last six weeks and one of these died of pneumonia and had

evidence of bone marrow depression. The other two dogs maintained a normal hematologic picture. Two dogs (including the one that died) showed reduced weight gain; the other two dogs that survived the dosage of 4 mg/kg/day showed at autopsy discoloured and mottled lungs but no histological abnormalities in the liver, spleen, kidneys, testes, adrenals, pancreas or myocardium. Bone marrows showed normal cellularity.

A dog given ten doses of 10 mg/kg, orally, over a 12-day period became moribund four days after the last dose and had agranulocytosis and acute ulcers of the anal and rectal region with tissue necrosis. At a dose of 7.5 mg/kg given orally for ten doses, a dog maintained its weight and showed a normal white blood cell count for several months after the study; the red blood cell count was slightly depressed to 3.7 million two weeks after the final dose, but the count gradually returned to normal. At a dose of 5 mg/kg for ten doses, a dog maintained its weight and continued to show a normal blood picture for several months. Dogs with kidney homografts generally tolerated doses of 10 mg/kg/day, orally, for two days followed by maintenance doses of 2.5 to 4 mg/kg/day.

The hepatotoxic potential of azathioprine was studied by Starzl *et al* in 18 normal dogs. Azathioprine alone was administered for 40 days in the same dosage as used for prevention of homograft rejection. There were declines in hematocrit, weight loss and elevations of SGOT, SGPT and alkaline phosphatase.

These changes tended to occur early suggesting that the liver injury was due to direct hepatotoxicity. Although there was usually a partial recovery from these biochemical abnormalities, 13 of the 18 dogs had histologic evidence of liver injury at the end of 40 days. The principal histologic alterations were usually in the centrilobular area. As Starzl pointed out, the hepatotoxicity of azathioprine is greater in dogs than in man. This is borne out by the 3% incidence of hepatitis in the cases reported in the Registry.

CARCINOGENICITY STUDIES

Rats

Azathioprine was administered orally in the diet at doses of 0, 3 or 10 mg/kg/day to groups of 70 male and 70 female Sprague-Dawley rats for 90 and 97 consecutive weeks, respectively.

A life table analysis indicated comparable cumulative survival of the control and 3 mg/kg/day female group. Survival of the male 3 mg/kg/day group began to diverge from the control group by day 600. Reduced cumulative survival of the male and female 10 mg/kg/day groups compared to the controls began by 450 and 350 days respectively. There were no effects on

food consumption. The mean weight of the 10 mg/kg group was lower than the untreated control group mean.

There was a marked depletion of body fat in the 10 mg/kg/day rats.

An increased incidence of neoplasms of the skin, ear canal (including the auditory sebaceous or Zymbal's gland) and preputial gland was associated with azathioprine administration. The presence of a few neoplasms of the nonglandular stomach in the treated males was considered potentially significant due to their rare spontaneous occurrence. Two mucinous adenocarcinomas of the duodenum, which were noted in the male 3 mg/kg/day group, were considered possibly significant.

Mice

A study was carried out to determine the carcinogenic effects of azathioprine when given orally in the diet to mice during an 18-month period. Six hundred (300 males and 300 females) clinically healthy 21-day-old mice were used in this study. Mice were randomly assigned to 1 of the 3 following dose groups of 100 males and 100 females: 0 mg/kg/ day, 3 mg/kg/day or 10 mg/kg/day.

Mice in the high dose group (10 mg/kg/day) were fed a drug-free diet during dose weeks 21 through 38 because high mortality due to drug toxicity was observed. Otherwise the drug-diet mixture was fed until there was 10 to 20% survival of that sex in any treatment group. Surviving females were sacrificed after 524 to 530 days on study and surviving males after 600 to 602 days on study.

Mice were observed daily and palpated weekly for tumours. Complete necropsies were performed on each mouse after death or sacrifice. Representative sections of all major organs and all tumours were fixed, prepared, and examined histologically from high dose (10 mg/kg/day) and control mice. Target organs and all tumours were examined from low dose (3 mg/kg/day) mice.

Azathioprine in the diet significantly reduced the survival of 3 mg/kg/day females and 10 mg/kg/day males and females. Paleness of the mucous membranes, probably due to anemia, was observed. Significant differences in food consumption and body weights were periodically observed, but they were not consistently present throughout the study.

The number of clinically palpable nodules was similar in control and treated mice. At necropsy enlarged thymuses, lymph nodes, and spleens were observed, especially in the high dose group. Cystic endometrial hyperplasia was present in the majority of control and treated females.

Histologically, both male and female mice had a dose-related increase ($p<0.01$) in lymphosarcomas. This increased incidence of lymphosarcoma in azathioprine-dosed females was also responsible for a significant ($p<0.01$) increase in total malignant and/or malignant plus benign tumours. In treated male mice, the incidence of malignant or malignant plus benign tumours was not significantly increased.

Synergistic immunosuppression with N-nitrosobutylurea and azathioprine induced leukemia, mean latent period of 189 days, in 14 of 24 (58%) C57BL mice. Immunosuppression with azathioprine of NZB X NZW mice that had lupus nephritis also increased the incidence of lymphosarcoma. In view of the above, lymphosarcoma, as observed in this current study in treated mice, may have been secondary to azathioprine immunosuppression.

An increased number of squamous cell carcinomas was observed in the preputial area of treated mice, and for purposes of statistical comparison were considered to be of preputial gland origin. Although the total number of these tumours in either treated group of male mice was not significantly greater than the number in controls, a positive dose response was detected statistically. The incidence of spontaneous preputial gland carcinomas reported in the literature is low; therefore, these tumours may have been induced by azathioprine.

TERATOLOGY STUDIES

Reproductive studies have been performed in a variety of species. The administration of azathioprine to pregnant rats and one strain of mice did not produce significant congenital anomalies. However, studies with pregnant rabbits and Swiss-Webster mice have shown that azathioprine has significant teratogenic potential producing resorptions and skeletal anomalies even when administered as late as the midpoint of gestation.

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